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i. Certain strains of Propionibacteria are able to produce significant amounts of NO under anaerobic conditions, which can further be accumulated in the surrounding medium.

ii. Synthesis of NO by Propionibacterium cultures does not involve the well known NO synthase pathway using arginine as a precursor, but another metabolic route using nitrate or nitrite as a substrate.

iii. NO synthesis by Propionibacteria can use nitrate already present in a YEL growth medium and can be further increased by the supply of nitrate and nitrite.

iv. NO synthesis by Propionibacteria is increased at physiological body temperature (37°C) relatively to temperatures normally used in vitro for growing bacteria.

v. A comparison between different strains of Propionibacteria showed that not all strains are able to produce NO, several being devoid of any capacity for NO synthesis. Some specific strains have been characterized as being able to produce much more NO than other strains.

6. Taking into consideration the overall comments from the Examiner relative to the applicant's invention, and more specifically his interpretation of previous published works,^{2,4,5} directly or indirectly related to Propionibacteria, it is clear that the book² published by Balows et al. (1992) and cited by the Examiner does not reveal or show experimental data demonstrating that Propionibacteria are able to produce NO. Chapter 23 entitled "The Denitrifying Prokaryotes," is the only chapter considering the production of NO molecules by bacteria. The only mention of Propionibacteria in this chapter (Page 556, second paragraph) is given as: "Whether a true respiratory utilization of nitrite by the fermentative Propionibacterium occurs, has been questioned; it may instead be a detoxification process (Kaspar, 1982)." This sentence does not explicitly mean or even suggest that Propionibacteria are able to synthesize NO. Moreover, if the Kaspar reference³ is analyzed, then it appears that no experimental data concerning the NO synthesis by Propionibacteria is given; the author considered *sensu stricto* the

² THE PROKARYOTES, edited by Balows et al., Springer-Verlag New York Inc., Vol. I, pages 554-556 and 834-849 (2d. ed. 1992).

³ Kaspar, H.F., *Nitrate Reduction to Nitrous Oxide by Propionibacteria: Detoxification Mechanism*, ARCH. MICROBIOL., 133:126-130 (1982).

synthesis of N_2O (nitrous oxide). Although it is well known that denitrifying bacteria have the metabolic apparatus to produce NO from nitrite, it cannot be deduced from the current knowledge that Propionibacteria share the same capacity. In chapter 37 of the Balows et al. reference², the genus Propionibacterium is described: the usual known habitat of such bacteria is limited to dairy products, while denitrifying bacteria are known to be telluric organisms. Therefore, in this book there is no direct or indirect evidence that can be used to suggest that Propionibacteria have the capacity to synthesize NO like denitrifying bacteria.

7. The second reference⁴ cited by the Examiner relates to a process to make flavored cheese, in which an inoculum of Propionibacteria can be used. No mention of NO, or nitrogen monoxide, synthesized by these bacteria or even by any other process can be found in the description of this patent. This can be further supported by the fact that despite detailed chemical analysis of bacteria proliferation media within 14 examples, the precursor compounds needed for NO synthesis (nitrate or nitrite) have never been quantified or even considered. Moreover, it cannot be concluded by any means, from this document that the consumption of cheese produced by the described procedure will release in the digestive tract significant amounts of Propionibacteria, and even so, that they will produce NO.

8. The third reference⁵ cited by the Examiner considers a process for counting Propionibacteria using a selective medium. Although considering extensively in the introduction section all the potential use of Propionibacteria, this patent does not invoke or even suggest the production of NO by these bacteria and its potential use. Moreover, the different propositions of selective media for Propionibacterium counting given in this patent do not consider explicitly the nitrogen sources, and more specifically the substrate needed (nitrate or nitrite) for NO synthesis.

9. Taken independently or even as a whole, none of the documents referred to by the Examiner provide any direct or indirect evidence to support the view that the invention proposed by applicants could be rendered obvious. None of them describes or even suggests (i) the synthesis of NO by Propionibacteria or uses a relevant reference to do so, or (ii) the use of these bacteria and their role in the digestive tract for NO accumulation. Overall, since the production

⁴ U.S. Patent No. 4,379,170 to Hettinga et al.

⁵ U.S. Patent No. 5,573,947 to Madec et al.

of NO by Propionibacteria and its potential accumulation have never been described under general (i.e., before their consumption) or post-consumption conditions, it cannot be deduced that there is the potential for NO production within the digestive tracts of consumers without the knowledge provided by the applicants.

10. Finally, none of the original results (see paragraph 5) that we obtained within the work carried out in collaboration with the applicants, and which constitute the basis of the proposed invention, can be rendered obvious from the analysis of the documents provided by the Examiner.

11. I further declare that all statements made herein of my own personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Date: JULY 25^H 2001

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